Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
664674		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2004/011480	10.08.2004	14.08.2003

International Patent Classification (IPC) or national classification and IPC

C12N15/09, C07K14/195, C12N9/16

Applicant

TAKARA BIO INC.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:

      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))

      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/JP Facsimile No.	Authorized officer Telephone No.

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Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (Rule 12.3 and 23.1(b))

☐ publication of the international application (Rule 12.4)

☐ international preliminary examination (Rule 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☒ the international application as originally filed/furnished

☐ the description:

pages _____ as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☐ the claims:

nos. _____ as originally filed/furnished

nos.* _____ as amended (together with any statement) under Article 19

nos.* _____ received by this Authority on _____

nos.* _____ received by this Authority on _____

☐ the drawings:

sheets _____ as originally filed/furnished

sheets* _____ received by this Authority on _____

sheets* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted the claims nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:
   - ☐ complied with.
   - ☒ not complied with for the following reasons:

Claims 1 to 19, 26 and 27 pertain to specific proteins that exhibit a dsRNA degrading activity, to a method for the production thereof, to compositions that comprise said specific proteins and to a method for degrading dsRNA by means of said compositions, etc. Therein, a number of claims make disclosures in relation to the coexistence of a protein that exhibits a nucleic acid binding activity or the fusion of a protein that exhibits a nucleic acid binding activity with a protein that exhibits a dsRNA degrading activity.

Meanwhile, claims 20 to 25, 28 and 29 pertain to an RNA synthesis method and the like, which are characterized in that a protein that exhibits an RNA synthesizing activity is combined with a protein that is capable of binding nucleic acids.

[Refer to the Supplemental Box]

4. Consequently, this report has been established in respect of the following parts of the international application:
   - ☒ all parts.
   - ☐ the parts relating to claims Nos. _____

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	3, 6, 8, 9, 14-16, 21, 23-25	YES
	Claims	1, 2, 4, 5, 7, 10-13, 17-20, 22, 26-29	NO
Inventive step (IS)	Claims		YES
	Claims	1-29	NO
Industrial applicability (IA)	Claims	1-29	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Citations

Document 1: P. Provost et al., "Ribonuclease activity and RNA binding of recombinant human Dicer," EMBO J., 2002, Vol. 21, No. 21, pages 5864 to 5874

Document 2: WO 01/68839 A2 (Cold Spring Harbor Laboratory), 21 September 2001

Document 3: WO 99/27117 A1 (Takara Shuzo Co., Ltd.), 03 June 1999

Document 4: W. BAE et al., "*Escherichia coli* CspA-family RNA chaperones are transcription antiterminators," Proc. Natl. Acad. Sci. USA, 2000, Vol. 97, No. 14, pages 7784 to 7789

Document 5: W. KREMER et al., "Solution NMR structure of the cold-shock protein from the hyperthermophilic bacterium *Thermatoga maritima*," Eur. J. Biochem., 2001, Vol. 268, pages 2527 to 2539

Document 6: Y. F. MELEKHOVETS et al., "Fusion with an RNA binding domain to confer target RNA specificity to an RNase: design and

engineering of Tat-RNase H that specifically recognizes and cleaves HIV-1 RNA *in vitro*," Nucl. Acids Res., 1996, Vol. 24, No. 10, pages 1908 to 1912

Explanations

1. Novelty

Claims 1, 2, 4, 5, 7, 10 to 13, 17 to 19, 26 and 27

Document 1 describes the production of a recombinant human Dicer protein which exhibits a dsRNA degrading activity, and indicates that said protein comprises a helicase domain, a PAZ domain, two RNase domains and a dsRNA binding domain upon the same peptide chain. Therein, the predicted amino acid sequence of the abovementioned protein overlaps with the sequences represented by SEQ ID NO: 4 and 17 set forth in the present application, and in particular overlaps with the entire length of the sequence that is represented by SEQ ID NO: 17.

In addition, document 1 further indicates that the abovementioned dsRNA binding domain actually exhibits a nucleic acid binding activity.

As a result, claims 1, 2, 4, 5, 7, 10 to 13, 17 to 19, 26 and 27 are not novel.

Claims 20, 22, 28 and 29

Document 4 indicates that the termination of transcription was inhibited and that constant genetic transcription was promoted in the presence of cold shock proteins from *E. coli*, which were overexpressed at a

temperature of 37°C.

As a result, claims 20, 22, 28 and 29 are not novel.

2. Inventive Step

Claims 3, 6, 8, 9 and 14 to 16

Document 1 compares the Dicer proteins from various organisms, and indicates that even the Dicer proteins which lack a PAZ domain (page 5866, left column, line 4) and the Dicer proteins which lack a dsRNA binding domain (page 5871, right column, lines 1 to 2) express a dsRNA degrading activity.

Such being the case, it would have been easy for a person skilled in the art to conceive of creating a protein that includes only two RNase domains as the smallest unit which is capable of expressing a dsRNA degrading activity, and creating proteins that include a PAZ domain and/or a dsRNA binding domain in addition to the two RNase domains. In addition, a person skilled in the art could also have selected the host or attempted to configure so that an appropriate protein (for example, the cold shock protein derived from *Thermatoga maritima* that is presented in document 5, which exhibits a DNA binding activity, or the like) is coexistent or fused with the abovementioned protein, as appropriate.

Furthermore, document 3 discloses a cold-inducible expression vector that exhibits an efficient expression level at normal temperatures; therefore, there cannot be considered to be significant technical difficulty involved in employing such a compound.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

In the description of the present application, the test results pertaining to the cold shock protein derived from *Thermatoga maritima* are expressed in abstract terms devoid of any objective disclosures involving specific comparative data or the like, and the description does not include any specific disclosures in relation to proteins (in particular, fusion proteins or the like) that exhibit a nucleic acid binding activity other than said specific cold shock proteins. As a result, the effects that result from the use of such proteins cannot be considered to be significant.

Such being the case, claims 3, 6, 8, 9 and 14 to 16 do not involve an inventive step.

Claims 21 and 23 to 25

It is well known that there are various cold shock proteins other than the cold shock proteins that are specifically presented in document 4, and it is thought that any of these cold shock proteins are capable of promoting transcription at low temperatures due to the fact that they exhibit a chaperone function. Consequently, it would have been easy for a person skilled in the art to conceive of attempting to efficiently synthesize RNA by means of various cold shock proteins. In addition, a person skilled in the art could also have attempted to employ the cold shock protein derived from *Thermatoga maritima* which is disclosed in document 5, as appropriate. Furthermore, a person skilled in the art could have selected which proteins that exhibit an RNA synthesizing activity can be used, as appropriate.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

In the description of the present application, the test results pertaining to the cold shock protein derived from *Thermatoga maritima* are expressed in abstract terms devoid of any objective disclosures involving specific comparative data or the like, and the description does not include any specific disclosures in relation to proteins (in particular, fusion proteins or the like) that exhibit a nucleic acid binding activity other than said specific cold shock proteins. As a result, the effects that result from the use of such proteins cannot be considered to be significant.

Such being the case, claims 21 and 23 to 25 do not involve an inventive step.

3. Industrial Applicability

Claims 1 to 29 exhibit industrial applicability.

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. Claim 3 claims a protein wherein the "functional domain of the Dicer comprises an RNAse IIIa domain, an RNAse IIIb domain and a dsRNA binding domain," whereas claim 4, which cites claim 3, claims a protein that also includes a PAZ domain.

Such being the case, the disclosures in question can be considered to be inconsistent, and thus unclear.

2. Claim 12 claims a "fusion protein." However, the description does not set forth any examples or the like wherein a functional fusion protein is created from a protein that exhibits a nucleic acid binding activity and the functional domain of the Dicer.

In addition, the assertion that configurations wherein a protein that exhibits a nucleic acid binding activity coexists with a protein that includes the functional domain of the Dicer and configurations wherein a protein that exhibits a nucleic acid binding activity is fused with a protein that includes the functional domain of the Dicer will exhibit similar effects is not supported by common technical knowledge, and is not sufficiently explained within the description.

Such being the case, the abovementioned "fusion protein" cannot be considered to be fully supported by the description.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☐ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Box IV.3

Therein, a number of proteins that are capable of binding nucleic acids and which contain the cold shock protein derived from *Thermotoga maritima* are well known to persons skilled in the art (if necessary, refer to the document Eur. J. Biochem., 2001, Vol. 268, pp. 2527 to 2539 or the document Proc. Natl. Acad. Sci. USA, 2000, Vol. 97, pp. 7784 to 7789, etc.). Therefore, the feature in question cannot be considered to be a special technical feature that is common to the abovementioned two groups of inventions.

As a result, there is no novel special technical feature which is common among the abovementioned two general inventive concepts, and thus the present international application cannot be considered to conform to the requirement of unity of invention (PCT Regulations, Rule 13 (sections 13.1, 13.2 and 13.3)).